06-02-00 A

CERTIFICATE OF EXPRESS MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service, Express Mail # EL492832119US, postage prepaid, in an envelope addressed to Box Patent Application, Assistant Commissioner for Patents, Washington, D.C. 20231-9999 on June 1, 2000.

June 1, 2000 Guy Powers

Guy Powers

EL492832119US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

REQUEST FOR FILING APPLICATION
Under Rule 53(a), (b) & (f)
(No Filing Fee or Oath/Declaration)
(Do NOT use for Provisional or PCT Applications)
Use for Design or Utility Applications

PATENT APPLICATION

RULE 53(f) NO DECLARATION

Assistant Commissioner of Patents
and Trademarks
Washington, DC 20231
Box Patent Application

Atty. Dkt. PMS-260755 (M#) | (Client Ref)

Date: June 1, 2000

Sir:

1. This is a Request for filing a new Patent Application ☐ Design ☒ Utility) entitled:

2. (Complete) Title: **ANTIFUNGAL AND ANTIMYCOBACTERIAL BASILISKAMIDES**

without a filing fee or Oath/Declaration but for which is enclosed the following:

3. ☒ Abstract 2 page(s).

4. 28 Pages of Specification (only spec. and claims); 5. ☐ Specification in non-English language

6. 15 Numbered claim(s); and

7. ☐ Drawings: _____ sheet(s) ☐ 1 set informal; 8. ☐ formal of size: ☐A4 ☐11"

9. **DOMESTIC/INTERNATIONAL** priority is claimed under 35 USC 119(e)/120/365(c) based on the following provisional, nonprovisional and/or PCT international application(s):

Application No.	Filing Date	Application No.	Filing Date
(1) 60/137,166	06/01/99	(2)	

10. **FOREIGN** priority is claimed under 35 USC 119(a)-(d)/365(b) based on filing in _____

Application No.	Filing Date	Application No.	Filing Date
(1)		(2)	

11. _____ (No.) Certified copy (copies): ☐ attached; ☐ previously filed (date) _____ in U.S. Application No. / _____ filed on _____

12. ☐ This is a reissue of Patent No. _____

13. ☐ See top first page re prior Provisional, National, International application(s) (X box only if info is there and do not complete corresponding item 14 or 15.)

14. ☐ **Amend the specification** by inserting before the first line -- This is a ☐ Continuation-in-Part ☐ Divisional ☐ Continuation ☐ Substitute Application (MPEP 201.09) of:

14(a) ☐ National Appln. No. / _____ filed .- - (M#)

14(b) ☐ International Appln. No. PCT/ _____ filed _____ which designated the U.S. - -

15. ☒ **Amend the specification** by inserting before the first line: --This application claims the benefit of U.S. Provisional Application No. 60/ 137,166 , filed 06/01/99.--

16. Extension to date: ☐ concurrently filed ☒ not needed ☐ previously filed

17. ☐ Prior application is assigned to

by Assignment recorded _____ Reel _____ Frame _____

18. ☐ **Attached: Preliminary Amendment**

19. This application is made by the following named inventor(s) **(Double check instructions for accuracy.):**

(1) Inventor	Michael	T.	**Kelly**
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	City	State/Foreign Country	Country of Citizenship
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(3) Inventor	Todd	A.	**Barsby**
	First	Middle Initial	Family Name
Residence	Vancouver, British Columbia	Canada	Canadian
	City	State/Foreign Country	Country of Citizenship
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20. NOTE: FOR ADDITIONAL INVENTORS, check box ☐ and attach sheet with same information regarding additional inventors.

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NOTE: File in duplicate with 2 post card receipts (PAT-103) & attachments

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Non Provisional Patent Application of	Group Art Unit: Unassigned
Michael T. Kelly, et al.	Examiner: Unassigned
Filed: June 1, 2000	**PRELIMINARY AMENDMENT**
For: ANTIFUNGAL AND ANTIMYCOBACTERIAL BASILISKAMIDES	

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June 1, 2000 Guy Powers

Guy Powers

Assistant Commissioner for Patents and Trademarks
Washington, D.C. 20231
Box Patent Application

Sir:

Entry of the following preliminary claim amendments is respectfully requested in the subject application. No new matter has been added.

In the Claims:

Please cancel claims 15 and 16.

Please amend claims 3, 7-11 and 14 as follows:

3. (Amended) The compound or physiologically acceptable salt thereof of claim 1 ~~or~~ 2 wherein Z' is a linear or branched, saturated or unsaturated one to eight carbon carbonyl optionally substituted with a substituent selected from the group consisting of: NH_2, NHR, NR_2, OH, OR, SH, SR, H and CF_3, wherein R is as defined.

7. (Amended) The compound or physiological salt thereof of ~~any one of claims 4-6~~ claim 4, wherein R_1 and R_2 are independently H or CH_3.

8. (Amended) The compound or physiological salt thereof of ~~any one of claims 4-6~~ claim 4, wherein R_3 is (a)

9. (Amended) The compound or physiological salt thereof of ~~any one of claims 4-6~~ claim 4, wherein X is NH_2.

10. (Amended) The compound or physiological salt thereof of ~~any one of claims 4-6~~ claim 4, wherein R_3 at C_7 is (a) and R_3 at C_9 is OH.

11. (Amended) The compound or physiological salt thereof of ~~any one of claims 4-6~~ claim 4, wherein R_3 at C_7 is OH and R_3 at C_9 is (a).

14. (Amended) A pharmaceutical composition comprising a compound or physiological salt thereof of ~~any one of claims 1-13~~ claim 1, and a pharmaceutically acceptable carrier.

Please add the following new claim:

--17. A pharmaceutical composition comprising a compound or physiological salt thereof of claim 4, and a pharmaceutically acceptable carrier.

Respectfully submitted,

Dated: June 1, 2000

By: _____
Georgina M. McPaul
Reg. No. 42,873

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ANTIFUNGAL AND ANTIMYCOBACTERIAL BASILISKAMIDES

Field of the Invention

This invention relates to polyketide amides having antibiotic activity.

Background of the Invention

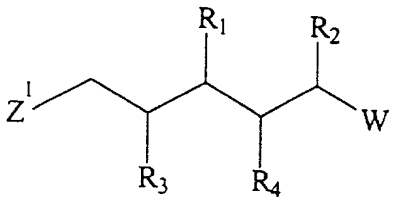
There is an urgent need for new antibiotics to treat pathogens that have developed resistance to antibiotics currently in use. Further, compounds that have antimycobacterial activity are rare. Compounds produced by marine microorganisms are being screened for antibiotic activity.

Japanese patent application 06-27802 published September 12, 1995 under No. 07238018 and entitled "Antimycotic Antibiotic Substance and its Production" discloses an antifungal compound YL-03709B-A obtained by fermentation of *Bacillus sp.* YL-03709B (FERM P-14126). The *Bacillus* was isolated from soils near Okinawa, Japan. The compound was reported as having antifungal activity against several organisms but low activity against *Candida albicans*, *Candida parapellosis*; *Saccharomyces cerevisiae*; *Saccharomyces sake*; and *Aspergillus niger* on Sabouraud/dextrose Agar medium.

An unidentified *Bacillus sp.* (MK-PNG-276A) was isolated from the tissues of a tubeworm collected in the tropical waters off Papau, New Guinea. Extracts from laboratory cultures of the latter organism exhibited broad spectrum antibiotic activity against a panel of antibiotic-resistant pathogens. Initial bioassay guided fractionation of crude extracts resulted in the isolation of the loloatins, a family of novel cyclic decapeptides (see PCT/CA97/00529). More recently, a class of novel polyketide amides were isolated from MK-PNG-276A cultures, which are termed basiliskamides herein. The basiliskamides have antibiotic activity.

Summary of the Invention

This invention provides a compound or a physiologically acceptable salt thereof, wherein the compound has the formula:

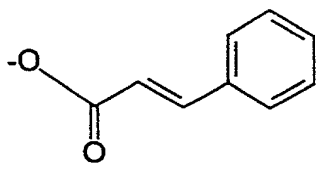


wherein:

R_1 and R_2 are the same or different and are independently H or R;

R is a structural fragment having a saturated or unsaturated linear, branched, or cyclic, skeleton containing one to ten carbon atoms in which the carbon atoms may be optionally substituted with a substituent selected from the group consisting of: -OH; =O; $-OR_5$; $-O_2CR_5$, -SH; $-SR_5$; $-SOCR_5$; $-NH_2$; $-NHR_5$; $-NH(R_5)_2$; $-NHCOR_5$; $NRCOR_5$; -I; -Br; -Cl; -F; -CN; $-CO_2H$; $-CO_2R_5$; -CHO; $-COR_5$; $-CONH_2$; $-CONHR_5$; $-CON(R_5)_2$; -COSH; $-COSR_5$; $-NO_2$; $-SO_3H$; $-SOR_5$; and $-SO_2R_5$, wherein R_5 is a linear, branched or cyclic, one to ten carbon saturated or unsaturated alkyl group;

R_3 and R_4 are different and are independently selected from the groups consisting of OH,

(a) 

and

(b) —O—Z—Ar

wherein,

Z^1 and Z are linear or branched, saturated or unsaturated. one to ten carbon fragments optionally substituted with Y;

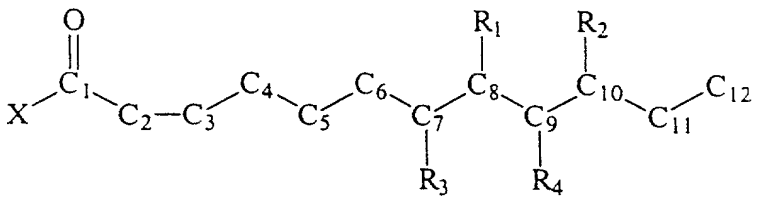
Ar is a monocyclic, bicyclic or tricyclic, fully or partially aromatic system containing five or six membered carbocyclic or, oxygen, nitrogen or sulphur containing heterocyclic rings. optionally substituted with R or Y;

Y is selected from the group consisting of: H; =O, -OH; -OR; $-O_2CR$; -SH; -SR; -SOCR; $-NH_2$; -NHR; $-NH(R)_2$; -NHCOR; NRCOR; -I; -Br; -Cl; -F; -CN- $-CO_2H$; $-CO_2R$; -CHO; -COR; $-CONH_2$; -CONHR; $-CON(R)_2$; -COSH; -COSR; $-NO_2$; $-SO_3H$; -SOR; $-SO_2R$; and, -O- (epoxide);

W is H or R;

with the provisos that when W is H, R_2 is not H; when R_2 is CH_3, W is not n-propyl; and, one of R_3 and R_4 is (a) or (b) and another of R_3 and R_4 is OH.

This invention also provides a compound or a physiologically acceptable salt thereof, wherein the compound has the formula:



wherein:

a single, double or triple bond exists between one or more of: C-2 and C-3; C-3 and C-4; C-4 and C-5; and, C-5 and C-6;

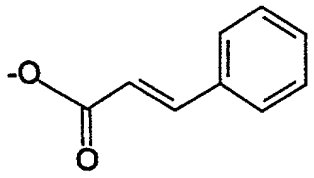
X is NH_2, NHR, NR_2, OH, OR, SH, SR, H, or CF_3;

R is a structural fragment having a saturated or unsaturated linear, branched, or cyclic, skeleton containing one to ten carbon atoms in which the carbon atoms may be optionally substituted with a substituent selected from the group consisting of: -OH; =O; $-OR_5$; $-O_2CR_5$; -SH; $-SR_5$; $-SOCR_5$; $-NH_2$; $-NHR_5$; $-NH(R_5)_2$; $-NHCOR_5$; $NRCOR_5$; -I; -Br; -Cl; -F; -CN; $-CO_2H$; $-CO_2R_5$; -CHO; $-COR_5$; $-CONH_2$; $-CONHR_5$; $-CON(R_5)_2$; -COSH; $-COSR_5$; $-NO_2$; $-SO_3H$; $-SOR_5$; and $-SO_2R_5$, wherein R_5 is a linear, branched or cyclic, one to ten carbon saturated or unsaturated alkyl group;

R_1 and R_2 are the same or different and are independently H or R;

R_3 and R_4 are different and are selected from the group consisting of: OH,

(a)



and

(b) —O—Z—Ar

wherein, Z is a linear or branched, saturated or unsaturated, one to ten carbon fragment optionally substituted with Y;

Ar is a monocyclic, bicyclic or tricyclic, fully or partially aromatic system containing five or six membered carbocyclic or, oxygen, nitrogen or sulphur containing heterocyclic rings, optionally substituted with R or Y;

Y is selected from the group consisting of: H; =O, -OH; -OR; -O_2CR; -SH; -SR; -SOCR; -NH_2; -NHR; -$NH(R)_2$; -NHCOR; NRCOR; -I; -Br; -Cl; -F; -CN- -CO_2H; -CO_2R; -CHO; -COR; -$CONH_2$; -CONHR; -$CON(R)_2$; -COSH; -COSR; -NO_2; -SO_3H; -SOR; -SO_2R; and, -O- (epoxide);

with the proviso that one of R_3 and R_4 is (a) or (b), and another of R_3 and R_4 is OH.

If a compound of this invention is naturally occurring (such as basiliskamide A or B as described herein) such a compound may be obtained from a natural source or may be sythnesized as described herein. In cases where such a naturally occurring compound is obtained from a natural source, the compound of this invention is characterized as being purified or partially purified. Thus, any compound of this invention that is naturally occurring will be substantially free of cellular contaminants. Cellular contaminants are defined as any component of a living cell (eg. proteins, nucleic acids, cell wall fragments, etc.) or a naturally occurring compound that is not a compound of this invention. The term "substantially free of cellular contaminants" means that a compound or a mixture of compounds of this invention, whether or not present in a pharmaceutical composition, will be present at a ratio of at least 3:1 (w/w) of the total amount of a compound or compounds of this invention to total amount of cellular contaminants present.

This invention also provides pharmaceutical compositions comprising a compound of this invention and a pharmaceutically acceptable carrier selected for the particular indication and mode of treatment in which the compound is to be used.

Compounds of this invention that are capable of forming salts may be in the form of a physiologically acceptable salt. Such a salt is any salt that is acceptable for use in pharmaceutical formulations. For example, where a compound of this invention has a carboxyl or sulphonic acid moiety, the counterion may be Na, K, Mg or Zn. Where the compound comprises a basic moiety such as an amine, the salt may be hydrochloride.

Pharmaceutical compositions of this invention will contain a compound or physiologically acceptable salt thereof in admixture with any carrier, excipient, dilutant, filler, thickener, etc., or in combination with any drug delivery moiety or device selected as suitable for a particular indication and mode of treatment desired. For example, pharmaceutical compositions of this invention may be formulated for injection (intravenous or otherwise), topical application, oral dosage (eg. tablets, capsules, powders), eye drops, aerosol delivery or cosmetic/cleansing formulations such as shampoos or skin cleansers. Selection of pharmaceutically acceptable carriers for compounds of this invention are within the knowledge of those of skill in the art. An example of a formulation for topical use is a creme-based formulation or carrier in which a compound of this invention or a pharmaceutically acceptable thereof is dissolved or emulsified.

This invention also provides a method for treatment of a patient (animal or human) afflicted with a fungal or mycobacterial infection comprising the administration to said patient of a therapeutically effective amount or a compound of pharmaceutical composition of this invention. This invention provides the use of a compound or pharmaceutical composition of this invention as an antifungal agent or as an antimycobacterial agent. The dose range of a compound of this invention will be selected in accordance with a particular indication or mode of treatment. Generally, the dose range will be between about 50 and about 500 mg/day for oral and intravenous applications and between about 0.5 and about 5 gram for topical applications.

Detailed Description of Embodiments of the Invention

Marine Bacterium MK-PNG-276A: The marine bacterium MK-PNG-276A was isolated during a collecting expedition off of Loloata Island, Papua New Guinea. MIDI analysis of cellular fatty acids indicated that MK-PNG-276A was an unknown species possibly within the genus *Bacillus*. MK-PNG-276A was deposited July 2, 1996 at the American Type Culture Collection (ATCC) under No. 55797.

Isolation of the Basiliskamides: The marine bacterium MK-PNG-276A was grown in moderate scale culture as confluent lawns for 5 days at 16 °C on trays of solid trypticase soy agar supplemented with NaCl to a final concentration of 1%. The cultures were harvested by gently scraping the cells from the agar surface. Bacterial cells (21.5 g dry weight) were immersed in and subsequently extracted with MeOH (3 X 250 mL) over a period of six days. Crude MeOH extracts showed broad spectrum antimicrobial activity against a variety of human pathogens, including methicillin resistant *Staphylococcus aureus*, *Eschericia coli*, *Candida albicans* and *Mycobacterium tuberculosis*.

The combined MeOH extracts were concentrated in vacuo and then partitioned between EtOAc (3 x 100 mL) and H_2O/MeOH (10:1 200 mL). The EtOAc extract was dried over anhydrous Na_2SO_4, filtered and reduced to dryness in vacuo to give 6.5 g of a gum. The gum was fractionated by Sephadex LH-20 chromatography (eluent MeOH) to give 226 mg of a fraction containing strongly UV absorbing compounds. This fraction was subsequently subjected to step gradient reversed-phase chromatography (eluent: 1:1 MeOH/H_2O to 100% MeOH) on a 10g Waters Sep-Pak. A strongly UV absorbing fraction (82 mg) was further separated into crude basiliskamide A and crude basiliskamide B (28 mg total) by a normal-phase silica gel flash chromatography (4:1 EtOAC/CH_2Cl_2). Final purification was accomplished by reversed-phase HPLC (7:3 MeOH/H_2O), yielding pure basiliskamides A (**1**, 14 mg) and B (**2**, 9 mg) as clear solids.

Structure Elucidation of Basiliskamides A (1) and B (2): Basiliskamide A (**1**) was isolated as a clear solid that gave a $[M + H]^+$ ion at m/z 386.23358 in the high resolution fast atom bombardment mass spectrum appropriate for a molecular formula

of $C_{23}H_{31}NO_4$. The ^{13}C NMR spectrum (Table 1) of basiliskamide A (**1**) showed only 21 well resolved resonances, indicating that there was an element of symmetry in the molecule. Resonances in the ^{1}H NMR spectrum of basiliskamide A were all well dispersed, which facilitated identification of the two major substructures.

A broad three proton ^{1}H NMR resonance at δ 7.40-7.41, that showed HMQC correlations to carbon resonances at δ 128.9 and 130.4, along with a broad two proton ^{1}H NMR resonance at δ 7.71, that showed HMQC correlations to a carbon resonance at δ 128.4, were all assigned to a monosubstituted phenyl ring. The phenyl ring accounted for the element of symmetry required by the ^{13}C NMR data. A one proton doublet at δ 7.65 in the ^{1}H NMR spectrum showed COSY correlations to the phenyl multiplet at δ 7.71 and to another one proton doublet at δ 6.61. The two doublets were assigned to a vinyl group that was the only substituent on the phenyl ring. HMBC correlations observed between the vinyl doublet resonance at δ 7.65 and the phenyl carbon resonance at δ 128.4 confirmed the attachment of the vinyl group to the phenyl ring. HMBC correlations observed between both of the vinyl proton resonances at δ 7.65 and 6.61 and a carbon resonance at δ 166.0, showed that the phenyl and vinyl fragments were part of a cinnamoyl residue. The vinyl protons had a vicinal scalar coupling of 16 Hz demonstrating the cinnamoyl residue had the E configuration.

Analysis of COSY, HMQC, and HMBC data collected for basiliskamide A (**1**) routinely identified the linear carbon chain extending from C-2 to C-12, including the positions of the $\Delta^{2,3}$ and $\Delta^{4,5}$ olefins, the methyl branches at C-8 and C-10, and the presence of -OR substituents at C-7 and C-9. HMBC correlations observed between both the H-2 and H-3 resonances at δ 5.55 and 6.31, respectively, and a carbon resonance at δ 167.5, showed that C-2 was attached to a carbonyl carbon. Only one nitrogen and two hydrogen atoms remained unaccounted for by the cinnamoyl and linear C-1 to C-12 chain fragments, suggesting that the C-1 carbonyl was a primary amide. A pair of broad one proton resonances at δ 6.82 and 7.32, that showed COSY correlations to each other but did not show HMQC correlations to carbon resonances, were assigned to the primary amide N<u>H</u> protons. The N<u>H</u> resonance at δ 6.82 showed an HMBC correlation to the C-2 resonance at δ 119.3, confirming the presence of the primary amide at the terminus of the linear C-1 to C-12 carbon chain. A COSY

correlation observed between an OH proton resonance at δ 4.57 and the H-7 resonance at δ 3.55 showed that there was an alcohol functionality at H-7 and, therefore, the cinnamoyl fragment had to be attached to the linear carbon chain via an ester linkage at C-9. An HMBC correlation observed between the H-9 methine resonance at δ 4.92 and the cinnamoyl carbonyl resonance at δ 166.0 confirmed the presence of the C-9 ester linkage.

H-2 and H-3 had a vicinal scalar coupling constant of 11 Hz typical of Z olefins, while H-4 and H-5 showed a 15 Hz vicinal coupling typical of E olefins. Difference nOe experiments confirmed the assigned olefinic configurations. Irradiation of the H-3 resonance at δ 6.31 induced an nOe in the H-2 resonance at δ 5.55 in agreement with the Z configuration for the $\Delta^{2,3}$ olefin. Similarly, irradiation of the H-5 resonance at δ 5.91 induced a strong nOe in the H-3 resonance at δ 6.31 supporting the E configuration for the $\Delta^{4,5}$ olefin.

The relative stereochemistry at C-7 and C-9 was determined by converting basiliskamide A (**1**) to the acetonide derivative **7**. Analysis of the HMQC data for **7**, showed that the acetonide methyl carbon resonances had chemical shifts of 19.8 and 30.4 ppm, typical of acetonides formed from *syn*-1,3-diols. Further analysis of the ^{1}H NMR data for the acetonide **7** showed that the dioxane ring existed in a chair conformation with the C-6 and C-10 carbons equatorial. A vicinal coupling constant of 10 Hz was observed between H-9 and H-8 indicating that H-8 was axial and, therefore, the C-14 methyl had to be equatorial, establishing the relative stereochemistries at C-7, C-8, and C-9 as shown in **7**. Standard Mosher ester methodology was used to show that C-7 in basiliskamide A (**1**) had the S configuration. The configuration of C-10 in **1** was not determined. However, basiliskamide A (**1**) is a homolog of YM47522 (**5**) and the absolute configuration at C-10 in **5** has been determined by synthesis to be R. Since the other chiral centers in **1** and **5** have identical configurations, and the and the ^{1}H and ^{13}C NMR data for **1** and **5** are nearly identical for the C-7, C-8, C-9 and C-10 centers, it is indicated that **1** also has the R configuration at C-10.

Basiliskamide B (**2**) was also isolated as a clear solid that gave a $[M + H]^+$ ion at m/z 386.23358 in the high resolution fast atom bombardment mass spectrum appropriate for a molecular formula of $C_{23}H_{31}NO_4$, identical to the formula of

basiliskamide A (**1**). Analysis of the 1D and 2D NMR data obtained for basiliskamide B (**2**) showed that it was simply an isomer of basiliskamide A, in which the cinnamoyl ester was at C-7 instead of C-9. Basiliskamide B (**2**) and basiliskamide A (**1**) were both converted to the same diol **6** by DIBAL reduction, demonstrating that both molecules had identical absolute configurations.

Basiliskamide A (1): isolated as a clear solid; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; IR (film) υ_{max}: 3348, 3205, 2966, 2934, 1705, 1697, 1635, 1595, 1450 cm^{-1}; UV (MeOH) λ_{max}: 262 nm (ε 41 000); $[\alpha]^{25}_{D}$ (MeOH) = -78 ; positive-ion HRFABMS $[M+H]^{+}$ *m/z* 386.23358 ($C_{23}H_{32}NO_4$, calcd 386.23313).

Basiliskamide B (2): isolated as a clear solid; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; IR (film) υ_{max}: 3348, 3205, 2962, 2926, 1702, 1664, 1637, 1595, 1450 cm^{-1}; UV (MeOH) λ_{max}: 262 nm (ε 43 000); $[\alpha]^{25}_{D}$ (MeOH) = -12 ; HREIMS $[M]^{+}$ *m/z* 385.22531 ($C_{23}H_{31}NO_4$, calcd 385.22531).

Reduction of Basiliskamides. To basiliskamides B (**2,,** 4.7 mg) in 1 mL THF under Ar (g), at -78 , 4 equivalents of diisobutylaluminum hydride (DIBAL-H) were added. The reaction was stirred overnight then diluted with EtOAc (3 mL) and quenched by the addition of 2 mL NH_4Cl (aq), stirring until the reaction mixture turned cloudy (10 min). The mixture was extracted thrice with EtOAc, and the combined organics were reduced to dryness in vacuo. Preparative normal-phase TLC (100 % EtOAc) followed by reversed-phas HPLC (70/30 MeOH/H_2O, 280 nm) gave 2 mg of **6**. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.44 (1H, dd, J = 15 Hz, 11Hz), 7.35 (1H, br s, NH), 6.86 (1H, br s, NH), 6.36 (1H, dd, J = 11 Hz), 6.02 (1H, dt, J = 15 Hz, 7 Hz), 5.57 (1H, d, J = 11 Hz), 4.48 (1H, d, J = 5.4 Hz, OH). 4.69 (1H, d, J = 4.4 Hz, OH), 3.80 (1H, m), 3.23 (1H, m), 2.26 (1H, m), 2.07 (1H, m), 1.61 (1H, m), 1.36 (1H, m), 1.35 (1H, m), 1.18 (1H, m), 0.84 (3H, t, J = 7 Hz), 0,72 (3H, d, J = 7 Hz), 0.66 (3H, d, J = 7 Hz); positive-ion HRFABMS $[M+H]^{+}$ *m/z* 256.19211 ($C_{14}H_{26}NO_3$, calcd 256.19127).

Formation of Acetonide (7). To 1.5 mg of **6** in 0.5 mL 2,2-dimethoxypropane, pyridinium p-toluenesulfonate (5 wt% diolbasiliskamide) was added. The reaction mixture was stirred under Ar (g) and heated at 60 C for 1 h.

The reaction mixture was filtered through silica (rinsed with EtoAc) and the solvents removed in vacuo. Reversed-phase HPLC (80/20 MeOH/H_2O) yielded 1 mg of **7**. ^1H NMR (400 MHz, DMSO-*d*6) δ 7.46 (1H, dd, *J* = 15 Hz, 11 Hz, H-4), 7.35 (1H, br s, NH), 6.85 (1H, br s, NH), 6.37 (1H, dd, *J* = 11 Hz, 11 Hz, H-3), 5.94 (1H, dt, *J* = 15 Hz, 7 Hz, H-5), 5.58 (1H, d, *J* = 11 Hz, H-2), 3.57 (1H, m, H-7), 3.48 (1H, dd, *J* = 10 Hz, 2 Hz, H-9), 2.44 (1H, m, H-6), 2.19 (1H, m, H-6'), 1.54 (1H, m, H-10), 1.36 (3H, s, Me-17), 1.33 (1H, m, H-8), 1.30 (1H, m, H-11), 1.25 (1H, m, H-11'), 1.23 (3H, s, Me-16), 0.83 (3H, t, *J* = 7 Hz, Me-12), 0.75 (3H, d, *J* = 7 Hz, Me-13), 0.71 (3H, d, *J* = 7 Hz, Me-14); ^{13}C NMR (DMSO-*d*6, 100 MHz) δ 167.8 (C-1), 140.6 (C-3), 138.5 (C-5), 128.9 (C-4), 120.2 (C-2), 97.6 (C-15), 74.9 (C-9), 74.0 (C-7), 36.5 (C-6), 35.1 (C-8), 34.6 (C-10), 30.4 (C-16), 26.7 (C-11), 19.8 (C-17), 12.7 (C-13), 12.1 (C-12), 11.6 (C-14); positive-ion HRFABMS $[M+H]^+$ *m/z* 296.22198, $C_{17}H_{30}NO_3$, calcd 296.2257.

Reaction of 1 with (*R*)-MTPA Acid. To a solution of **1** (1.5 mg) in 0.5 mL dry CH_2Cl_2 were added DMAP (1 mg), a drop of triethylamine and (*R*)-MTPA acid (4 mg) and the solution stirred for 16 h. Removal of solvent in vacuo, followed by preparative reversed-phase TLC (100% MeOH), then reversed-phase HPLC (MeOH/H2O 4:1) gave the (*R*)-MTPA ester **1a** (0.8 mg). ^1H NMR (500 MHz, DMSO-*d*6) δ 7.72 (3H, br envelope), 7.43 (9H, br envelope), 7.35 (1H, br s), 6.84 (1H, br s), 6.70 (1H, d, *J* = 16 Hz), 6.20 (1H, dd, *J* = 11 Hz, 11 Hz), 5.58 (2H, m), 5.17 (1H, m), 4.98 (1H, m), 3.43 (3H, s), 2.60 (1H, m), 2.26 (2H, br m), 1.72 (1H, m), 1.29 (2H, br m), 1.17 (1H, m), 0.95 (3H, d, *J* = 7 Hz), 0.93 (3H, d, *J* = 7 Hz), 0.88 (3H, t, *J* = 7 Hz); positive-ion HRFABMS $[M+H]^+$ *m/z* 602.27148, $C_{33}H_{39}NO_6F_3$, calcd 602.272950.

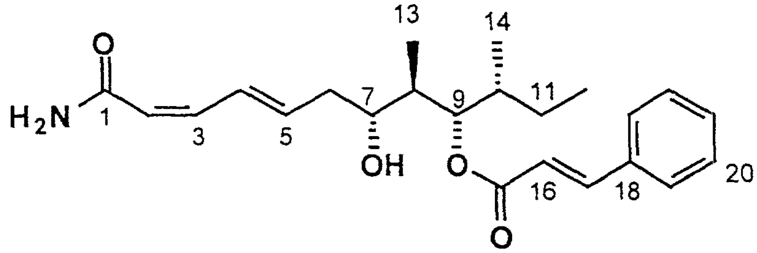
Reaction of 1 with (*S*)-MTPA Acid. To a solution of **1** (1.5 mg) in 0.5 mL dry CH_2Cl_2 were added DMAP (1 mg), a drop of triethylamine and (*S*)-MTPA acid (4 mg) and the solution stirred for 16 h. The reaction was quenched and purified as above, yielding the (*S*)-MTPA ester **1b** (0.4 mg). ^1H NMR (500 MHz, DMSO-*d*6) δ 7.72 (3H, br envelope), 7.54 (1H, m), 7.43 (8H, br envelope), 7.38 (1H, br s), 6.91 (1H, br s), 6.72 (1H, d, *J* = 16 Hz), 6.36 (1H, dd, *J* = 11 Hz, 11 Hz), 5.78 (1H, m),

5 5.64 (1H, d, J = 11 Hz), 5.13 (1H, m), 4.94 (1H, m), 3.42 (3H, s), 2.63 (1H, br m), 2.35 (1H, m), 2.17 (1H, m), 1.65 (1H, m), 1.27 (2H, br m), 1.15 (1H, m), 0.89 (3H, d, J = 7 Hz), 0.87 (3H, t, J = 7 Hz), 0.70 (3H, d, J = 7 Hz); positive-ion HRFABMS $[M+H]^+$ *m/z* 602.27352, $C_{33}H_{39}NO_6F_3$, calcd 602.272950.

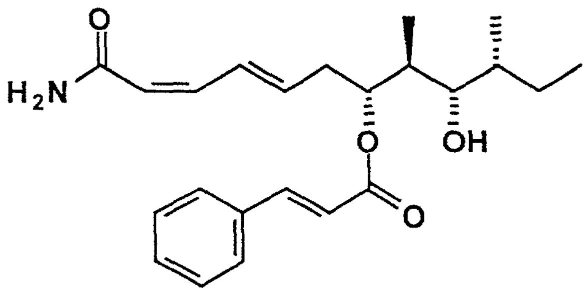
Table 1. ^{13}C (100 MHz) and ^{1}H (500 MHz) NMR Spectal Data for Basiliskamides A and B in DMSO-d_6

	Basiliskamide A (1)		Basiliskamide B (2)	
Atom	$\delta\ ^{13}C$	$\delta\ ^{1}H$ (intgrn, m, *J*(Hz))	$\delta\ ^{13}C$	$\delta\ ^{1}H$ (intgrn, m, *J*(Hz))
1	167.5		167.4	
2	119.3	5.55 (1H, d, 11)	119.9	5.57 (1H, d, 11)
3	140.5	6.31 (1H, dd, 11, 11)	140.0	6.33 (1H, dd, 11, 11)
4	128.2	7.40 (1H, m)	128.8	7.51 (1H, dd, 15, 11)
5	140.5	5.91 (1H, dt, 15, 7)	138.0	5.87 (1H, dt, 15, 7)
6	34.7	2.28 (1H, m)	31.8	2.53 (1H, m)
6'		1.99 (1H, m)		2.36 (1H, m)
7	69.6	3.49 (1H, m)	73.0	5.40 (1H, dt, 10.5, 3)
8	40.7	2.03 (1H, m)	39.4	1.92 (1H, m)
9	76.3	4.92 (1H, dd, 9.5, 2)	74.0	3.26 (1H, m)
10	35.5	1.67 (1H, m)	36.3	1.40 (1H, m)
11	26.4	1.25 (1H, m)	26.5	1.38 (1H, m)
11'		1.11 (1H, m)		1.21 (1H, m)
12	10.1	0.87 (3H, t, 7.5)	11.8	0.85 (3H, t, 7)
13	11.6	0.84 (3H, d, 7)	10.7	0.83 (3H, d, 7)
14	12.8	0.90 (3H, d, 7)	12.1	0.74 (3H, d, 7)
15	166.0		165.5	
16	118.0	6.61 (1H, d, 16)	118.5	6.59 (1H, d, 16)
17	144.6	7.65 (1H, d, 16)	144.1	7.60 (1H, d, 16)
18	134.0		134.0	
19	128.4	7.71 (2H, m)	128.2	7.70 (2H, m)
20	128.9	7.41 (2H, m)	129.0	7.40 (2H, m)
21	130.4	7.40 (1H, m)	130.2	7.40 (1H, m)
NH_2		7.31, 6.83 (2H, s)		7.34, 6.86 (2H, s)
OH		4.57 (1H, d, 5)		4.48 (1H, m)

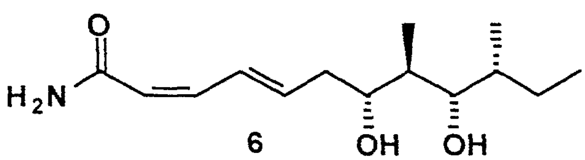
Structures of Basilikamides and Derivatives



Basiliskamide A



Basiliskamide B



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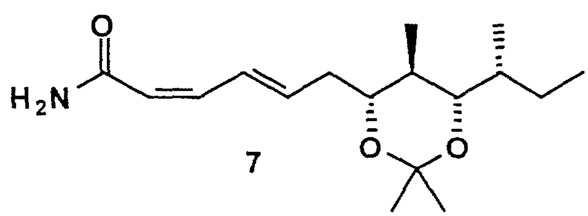

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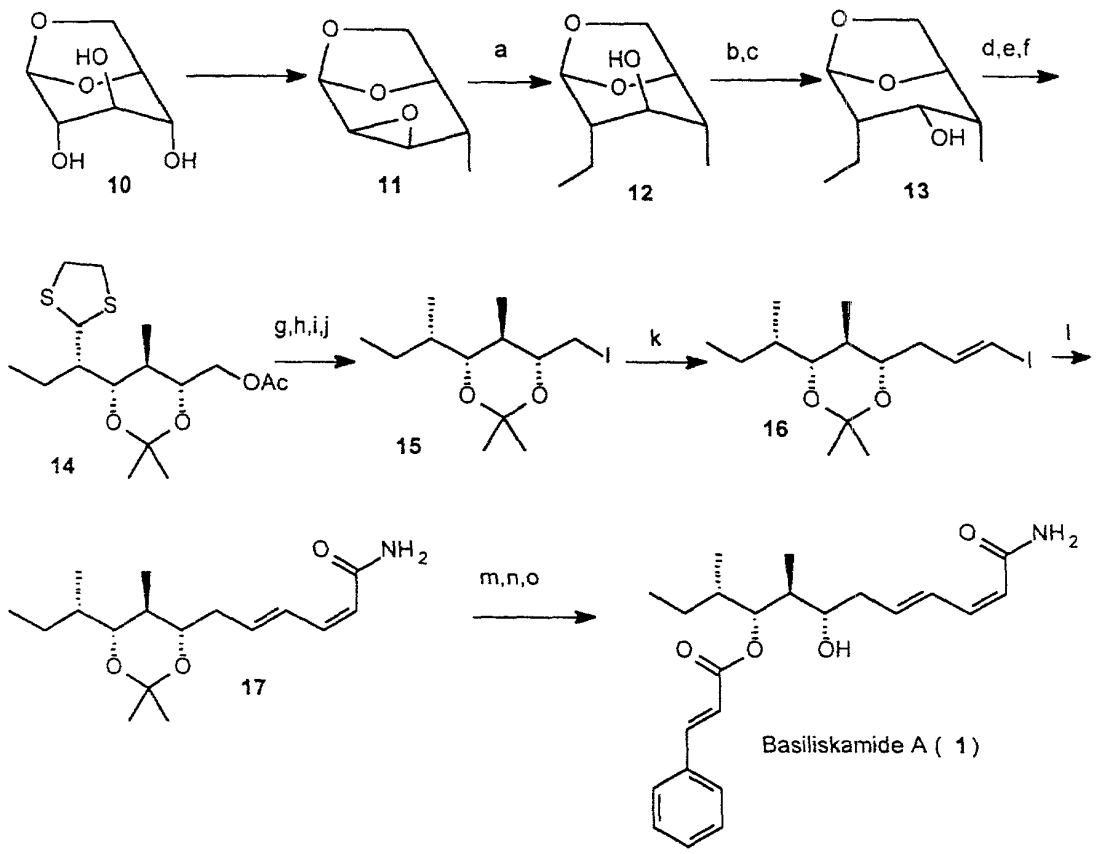
Preparation of Basiliskamides: Compounds of this invention may be prepared from a natural source by fermentation as described above, or by total synthesis, for example by modification of the total synthesis of YM47522 that was described in Ermokenko, M.S. Tetrahedron Letters, **1996**, *37*, 6711-12 (as exemplified in the scheme below for basiliskamide A).



a) $(CH_3CH_2)_2Mg/Et_2O$, Δ, 0.5 h;

a) NMO-Pr_4NRuO_4 (0.02 eq), MS 4 Å/ MeCN, rt, 0.5 h;

b) $NaBH_4$-$CeCl_3 \cdot 7H_2O$/MeOH, -20 C, 0.5 h;

c) $HSCH_2CH_2SH$, $BF_3.Et_2O/CH_2Cl_2$, rt, 2 h;

d) Ac_2O/Py, -10 C, 2h;

e) Me_2CO-$Me_2C(OMe)_2$, TsOH (cat);

f) Ra-Ni/EtOH, Δ, 0.5 h;

g) K_2CO_3/MeOH, rt, 0.5 h

h) TsCl/Py;

i) LiI-HMPA/PhMe, Δ, 0.5 h;

j) Me_2CuLi-LiCN, 2 eq (E)-Bu_3SnCH=CHSnBu_3/THF-Et_2O, rt, 2 h, then **15**, -78 C to rt, then NIS, rt;

k) (Z)-Bu_3SnCH=$CHCONH_2$, $(MeCN)_2PdCl_2$ (0.05 eq)/DMF, rt, 24 h;

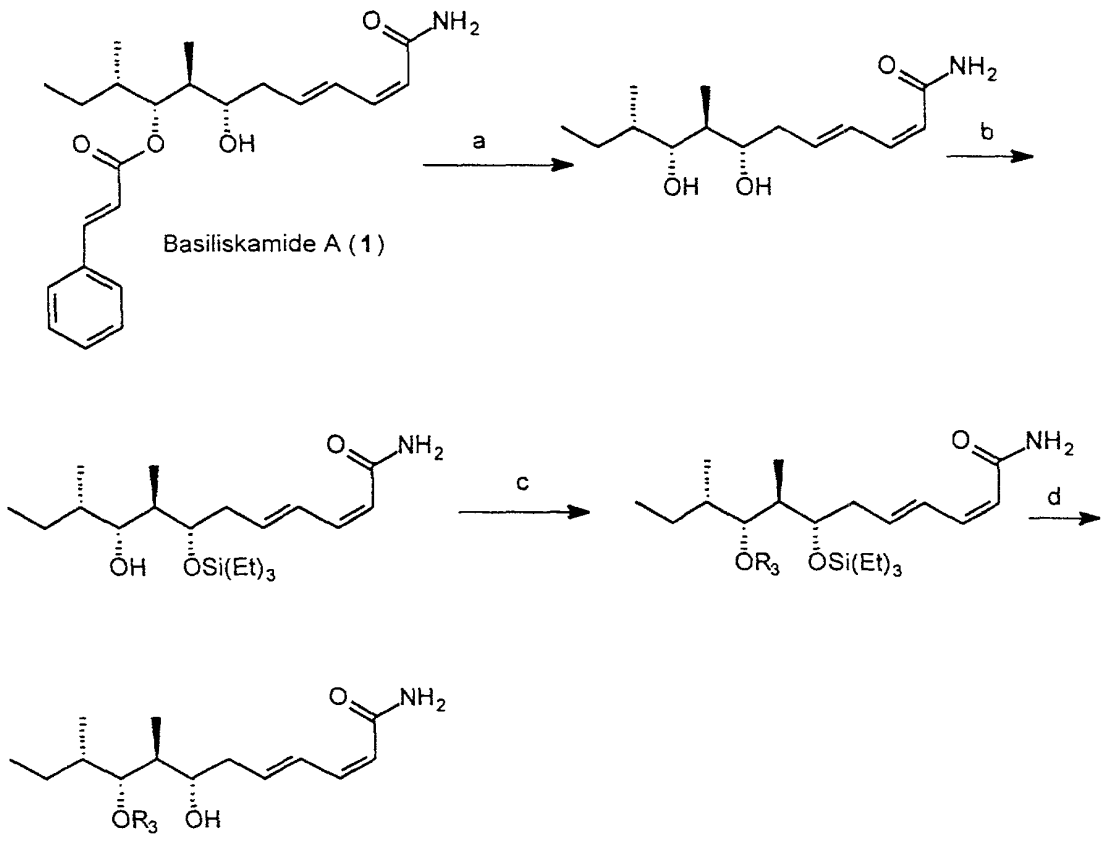
l) AcOH-H_2O (4:1), 60 C, 6 h;

m) 1.2 eq Et_3SiCl/Py, 0 C, then PhCH=CHCOCl, DMAP/CH_2Cl_2, rt;

n) HF (aq)-MeCN, rt, 1 h.

Conversion of compound 10 to compound 11 is as described in Sviridov, A. F. et al. *Izv. Akad. Nauk SSR, Ser. Khim.* **1982**, 2572-2574.

Analogs can be prepared by modification of the total synthesis shown above or by semisynthesis from a product of the total synthesis or a naturally derived product. An example employing basiliskamide A (**1**) is shown in the scheme below.



a) DIBAL (4 eq), THF, -78 C, 15h

b) 1.2 eq Et_3SiCl/Py, 0 C

c) alkylate or acylate alcohol (i.e for acylation ArCH=CHCOCl, DMAP/CH_2Cl_2, rt)

d) HF (aq)-MeCN, rt, 1 h

Antifungal Activity of the Basiliskamides: The antifungal activity of basiliskamides A and B was compared to that of the known antifungal agent, amphotericin B. A standardized macrobroth dilution method as used which was developed and published by the National Committee for Clinical Laboratory Standards (Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard 1996 M27-A, Vol. 15, No.10). An agar method based on the standardized broth method was also used. The results are presented in Tables 2-5 below.

Table 2. Antifungal Activity of Basiliskamide A and B Tested by Macrobroth Dilution

	Minimal Inhibitory Concentration (μg/ml)		
Basiliskamide	*Candida albicans*	*Trichophyton rubrum*	*Aspergillus fumigatus*
A	0.5	1.0	4.0
B	≥32	4.0	≥32

Table 3. Antifungal Activity of Basiliskamide A and B Tested by Agar Dilution

	Minimal Inhibitory Concentration (μg/ml)		
Basiliskamide	*Candida albicans*	*Trichophyton rubrum*	*Aspergillus fumigatus*
A	1.0	0.5	2.5
B	3.1	5.0	5.0

Table 4. Activity of Basiliskamide A Compared to Amphotericin B Against 7 Clinical Isolates of *Candida albicans* as Determined by Macrobroth Dilution

Isolate Number	Minimal Inhibitory Concentration (μg/ml) Basiliskamide A	Amphotericin B
8167	0.5	0.5
8362	0.5	0.5
8363	0.5	0.5
8364	0.5	0.5
8365	0.5	0.5
8366	0.5	0.5
8367	0.5	0.5

Table 5. Comparative Activity of Basiliskamides A, B and YL-03709B-A as Determined by Agar Dilution

Target Organism	Minimal Inhibitory Concentration (μg/ml) Basiliskamide A	Basiliskamide B	Reported Value for YL-03709B-A
Candida albicans	1.0	3.1	25
Aspergillus fumigatus	2.5	5.0	≥50

These findings demonstrate that the basiliskamides have superior antifungal activities and are active against the dermatophyte, *Trichophyton rubrum*, the yeast, *Candida albicans*, and the opportunistic fungus, *Aspergillus fumigatus*. Basiliskamide A activity against clinical isolates of the yeast, *Candida albicans* when tested by the broth dilution method is comparable to that of amphotericin B, a commonly used antifungal agent. The data presented in Table 5 demonstrates that Basiliskamide A is about 25 x more active than YL-03709B-A against the yeast *Candida albicans* and the filamentous fungus *Aspergillus fumigatus*, in view of the information for YL-

03709B-A reported in Japanese patent application 06-27802. Basiliskamide B also is significantly more active against these organisms than YL-03709B-A.

Antimycobacterial Activity of the Basiliskamides: The Basiliskamides were tested for antimycobacterial activity using a standardized agar dilution method (Underlied, CB and Salfinger, S. 1995. In Manual of Clinical Microbiology, Murray, Baron, Pfaller, Tenover, Yolken (Eds.), ASM Press, page 1395-1404). Activity of basiliskamide A, basiliskamide B, and acylated derivatives of the two compounds were tested for activity against *Mycobacterium tuberculosis*, the cause of tuberculosis, and *Mycobacterium avium-intracellulare*, an important cause of mycobacterial infections in immunocompromized patients such as those with AIDS. The results are shown in Table 6.

Table 6. Antimycobacterial activity of the Basiliskamides

	Minimal Inhibitory Concentration (μg/ml)	
Compound	*M. tuberculosis*	*M. avium-intracellulare*
Basiliskamide A	25	100
Basiliskamide B	50	>100
Acylated A (2-16)	>100	>100
Acylated B (2-16)	>100	≥ 50

These results indicate that basiliskamide A and B each have activity against *M. tuberculosis*. Basiliskamide A has activity against *M. avium-intracellulare*, but basiliskamide B appears to be relatively inactive against this organism. Decreasing the number of carbons in the backbone of the molecule may increase activity. Such is the case with the increased antifungal activity of the basiliskamides (A and B) that have one less carbon in the molecule's backbone than YL-03709B-A.

Cytotoxicity Testing of Basiliskamide A: Serial dilutions of basiliskamide A were prepared in cell culture medium and tested for toxicity for normal human fibroblast cells and for human tumor cell line. The effect of basiliskamide (basil) was compared to that of the known antifungal compound amphotericin B (ampho). The appearance of the cells was assessed after 48 hours exposure to the compounds. The results are shown in Table 7. Basiliskamide produced no cytotoxicity for normal human fibroblast cells at concentrations less than 100 μg/ml compared to amphotericin B (ampho) which was toxic at concentrations as low as 25 μg/ml. Against human tumor cells, basiliskamide showed minor toxicity at concentrations above 3 μg/ml. These findings suggested that basiliskamide is less toxic for normal human cells than the widely used amphotericin B.

Table 7 Basiliskamide Cytoxicity Testing

Cytopathic Effect (48 hours)				
	Human Fibroblast		Human Tumor	
Conc (μg/ml	Basil	Ampho	Basil	Ampho
100	2	4	4	4
50	0	2	1	3
25	0	2	1	1
12.5	0	1	1	0
6.25	0	0	2	0
3.12	0	0	2	0
1.57	0	0	0	0
0.78	0	0	0	0
0	0	0	0	0

1 - slight change in morphology vs. control

2 - occasional rounding, vacuolization, or granularity

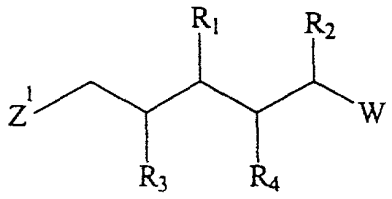
3 - rounding, vacuolization, detachment of 50% of cells

4 - destruction of monolayer

All publications, patents and patent applications referred to herein are hereby incorporated by reference. While this invention has been described according to particular embodiments and by reference to certain examples, it will be apparent to those of skill in the art that variations and modifications of the invention as described herein fall within the spirit and scope of the attached claims.

WE CLAIM:

1. A compound or a physiologically acceptable salt thereof, wherein the compound has the formula:

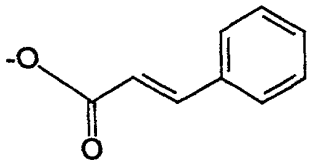


wherein:

R_1 and R_2 are the same or different and are independently H or R;

R is a structural fragment having a saturated or unsaturated linear, branched, or cyclic, skeleton containing one to ten carbon atoms in which the carbon atoms may be optionally substituted with a substituent selected from the group consisting of: -OH; =O; $-OR_5$; $-O_2CR_5$, -SH; $-SR_5$; $-SOCR_5$; $-NH_2$; $-NHR_5$; $-NH(R_5)_2$; $-NHCOR_5$; $NRCOR_5$; -I; -Br; -Cl; -F; -CN; $-CO_2H$; $-CO_2R_5$; -CHO; $-COR_5$; $-CONH_2$; $-CONHR_5$; $-CON(R_5)_2$; -COSH; $-COSR_5$; $-NO_2$; $-SO_3H$; $-SOR_5$; and $-SO_2R_5$, wherein R_5 is a linear, branched or cyclic, one to ten carbon saturated or unsaturated alkyl group;

R_3 and R_4 are different and are independently selected from the groups consisting of OH,

(a) 

and

(b) —O—Z—Ar

wherein,

Z^1 and Z are linear or branched, saturated or unsaturated, one to ten carbon fragments optionally substituted with Y;

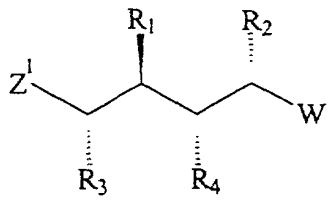
Ar is a monocyclic, bicyclic or tricyclic, fully or partially aromatic system containing five or six membered carbocyclic or, oxygen, nitrogen or sulphur containing heterocyclic rings, optionally substituted with R or Y;

Y is selected from the group consisting of: H; =O, -OH; -OR; $-O_2CR$; -SH: -SR; -SOCR; $-NH_2$; -NHR; $-NH(R)_2$; -NHCOR; NRCOR; -I; -Br; -Cl; -F; -CN- $-CO_2H$; $-CO_2R$; -CHO; -COR; $-CONH_2$; -CONHR; $-CON(R)_2$; -COSH; -COSR; $-NO_2$; $-SO_3H$; -SOR; $-SO_2R$; and, -O- (epoxide);

W is H or R;

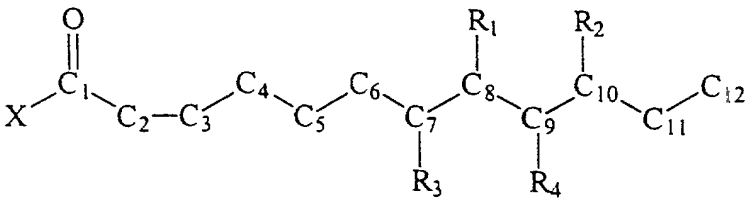
with the provisos that when W is H, R_2 is not H; when R_2 is CH_3, W is not n-propyl; and, one of R_3 and R_4 is (a) or (b) and another of R_3 and R_4 is OH.

2. The compound or physiologically acceptable salt thereof of claim 1 having the stereoisomeric form:



3. The compound or physiologically acceptable salt thereof of claim 1 or 2 wherein Z^1 is a linear or branched, saturated or unsaturated one to eight carbon carbonyl optionally substituted with a substituent selected from the group consisting of: NH_2, NHR, NR_2, OH, OR, SH, SR, H and CF_3, wherein R is as defined.

4. A compound or a physiologically acceptable salt thereof, wherein the compound has the formula:



wherein:

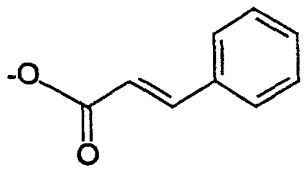
a single, double or triple bond exists between one or more of: C-2 and C-3; C-3 and C-4; C-4 and C-5; and, C-5 and C-6;

X is NH_2, NHR, NR_2, OH, OR, SH, SR, H, or CF_3;

R is a structural fragment having a saturated or unsaturated linear, branched, or cyclic, skeleton containing one to ten carbon atoms in which the carbon atoms may be optionally substituted with a substituent selected from the group consisting of: -OH; =O; $-OR_5$; $-O_2CR_5$, -SH; $-SR_5$; $-SOCR_5$; $-NH_2$; $-NHR_5$; $-NH(R_5)_2$; $-NHCOR_5$; $NRCOR_5$; -I; -Br; -Cl; -F; -CN; $-CO_2H$; $-CO_2R_5$; -CHO; $-COR_5$; $-CONH_2$; $-CONHR_5$; $-CON(R_5)_2$; -COSH; $-COSR_5$; $-NO_2$; $-SO_3H$; $-SOR_5$; and $-SO_2R_5$, wherein R_5 is a linear, branched or cyclic, one to ten carbon saturated or unsaturated alkyl group;

R_1 and R_2 are the same or different and are independently H or R;

R_3 and R_4 are different and are selected from the group consisting of: OH,

(a) 

and

(b) —O—Z—Ar

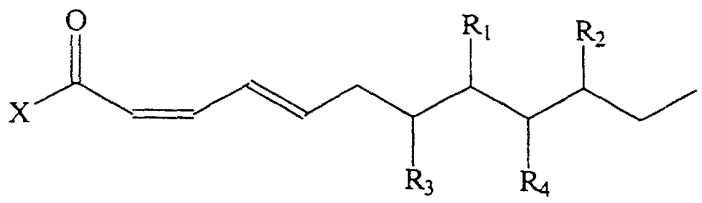
wherein, Z is a linear or branched, saturated or unsaturated, one to ten carbon fragment optionally substituted with Y;

Ar is a monocyclic, bicyclic or tricyclic, fully or partially aromatic system containing five or six membered carbocyclic or, oxygen, nitrogen or sulphur containing heterocyclic rings, optionally substituted with R or Y;

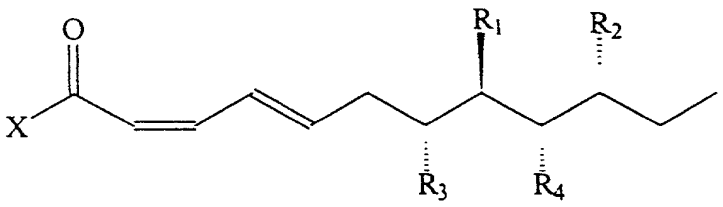
Y is selected from the group consisting of: H; =O. -OH; -OR; -O_2CR; -SH; -SR; -SOCR; -NH_2; -NHR; -$NH(R)_2$; -NHCOR; NRCOR: -I; -Br; -Cl; -F; -CN- -CO_2H; -CO_2R; -CHO; -COR; -$CONH_2$; -CONHR; -$CON(R)_2$; -COSH; -COSR; -NO_2; -SO_3H; -SOR; -SO_2R; and, -O- (epoxide);

with the proviso that one of R_3 and R_4 is (a) or (b). and another of R_3 and R_4 is OH.

5. The compound or physiologically acceptable salt thereof of claim 4 having the structure:



6. The compound or physiologically acceptable salt thereof of claim 4, having the structural and stereoisomeric form:



7. The compound or physiological salt thereof of any one of claims 4-6, wherein R_1 and R_2 are independently H or CH_3.

8. The compound or physiological salt thereof of any one of claims 4-7, wherein R_3 is (a).

9. The compound or physiological salt thereof of any one of claims 4-8, wherein X is NH_2.

10. The compound or physiological salt thereof of any one of claims 4-9, wherein R_3 at C_7 is (a) and R_3 at C_9 is OH.

11. The compound or physiological salt thereof of any one of claims 4-9, wherein R_3 at C_7 is OH and R_3 at C_9 is (a).

12. A compound according to claim 4, wherein the compound is Basiliskamide A substantially free of cellular contaminants.

13. A compound according to claim 4, wherein the compound is Basiliskamide B substantially free of cellular contaminants.

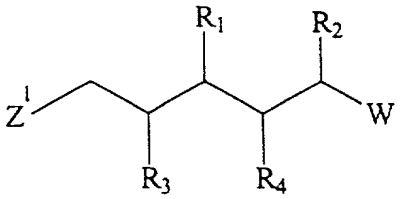
14. A pharmaceutical composition comprising a compound or physiological salt thereof of any one of claims 1-13, and a pharmaceutically acceptable carrier.

15. The use of a compound or physiological salt thereof of any one of claims 1-13. as an antifungal agent.

16. The use of a compound or physiological salt thereof of any one of claims 1-3. as an antimycobacterial agent.

ABSTRACT

Antibiotic polyketide compounds are provided having the formula

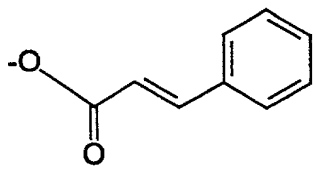


wherein:

R_1 and R_2 are the same or different and are independently H or R;

R is a structural fragment having a saturated or unsaturated linear, branched, or cyclic, skeleton containing one to ten carbon atoms in which the carbon atoms may be optionally substituted with a substituent selected from the group consisting of: -OH; =O; $-OR_5$; $-O_2CR_5$, -SH; $-SR_5$; $-SOCR_5$; $-NH_2$; $-NHR_5$; $-NH(R_5)_2$; $-NHCOR_5$; $NRCOR_5$; -I; -Br; -Cl; -F; -CN; $-CO_2H$; $-CO_2R_5$; -CHO; $-COR_5$; $-CONH_2$; $-CONHR_5$; $-CON(R_5)_2$; -COSH; $-COSR_5$; $-NO_2$; $-SO_3H$; $-SOR_5$; and $-SO_2R_5$, wherein R_5 is a linear, branched or cyclic, one to ten carbon saturated or unsaturated alkyl group;

R_3 and R_4 are different and are independently selected from the groups consisting of OH,

(a) 

and

(b) —O—Z—Ar

wherein.

Z^1 and Z are linear or branched, saturated or unsaturated, one to ten carbon fragments optionally substituted with Y;

Ar is a monocyclic, bicyclic or tricyclic, fully or partially aromatic system containing five or six membered carbocyclic or, oxygen, nitrogen or sulphur containing heterocyclic rings, optionally substituted with R or Y;

Y is selected from the group consisting of: H; =O, -OH; -OR; $-O_2CR$; -SH; -SR; -SOCR; $-NH_2$; -NHR; $-NH(R)_2$; -NHCOR; NRCOR; -I; -Br; -Cl; -F; -CN- $-CO_2H$; $-CO_2R$; -CHO; -COR; $-CONH_2$; -CONHR; $-CON(R)_2$; -COSH; -COSR; $-NO_2$; $-SO_3H$; -SOR; $-SO_2R$; and, -O- (epoxide);

W is H or R;

with the provisos that when W is H, R_2 is not H; when R_2 is CH_3, W is not n-propyl; and, one of R_3 and R_4 is (a) or (b) and another of R_3 and R_4 is OH.